Week 1

June 6, 2016

What We Did Today:
Made Liquid LB Media
Worked on Touch Tomorrow Posters
Streaked Florescent Plates

LB Prep Protocol
Add 25g LB mix per Liter. Mix well, then autoclave for an hour and a half.

General Lab
Creation of LB media with 1x CAM antibiotic, creation of 50% Glycerol, creation of Deionized Water samples -MRG

Igem Preparation
Creation of LB agar plates with CAM antibiotic
Transformed GFP and RFP genes into *E.coli* cells -MRG

Ginny and Jingyi:
Tested Extractions with banana and strawberry
• DNA extraction liquid
• DNA extraction trials: bananas
    - About 1 inch length

    Results: The liquid would not drain; no DNA extracted

June 6, 2016

What we did today:
Learned mammalian cell culture
Thawed cells and plated
Made Media
Worked on poster and survey
Made preps for strawberry extraction
Built dark box

Spray hood down with ethanol, wipe from back to front, turn on UV light for a few minutes

Making media:
We made 100mL of media using: FBS (10% of final concentration), Glutamine (1% of final concentration), PS (1% of final concentration), and DMEM (88% of final concentration)
For us this time, that meant using 5 mL FBS, 0.5 mL Glut, 0.5 mL PS, and 44 mL DMEM

Thawing Cells Protocol:
5ml media then draw up the cell line into the same pipette. Slowly dispense into final tube. Mix lightly, let rest for a few minutes. Run through centrifuge at 800 RPM for 5 minutes
Pipette as much of it as you can off without disturbing the pellet of cells at the bottom of the tube. Dispose of old media in sterilized beaker
Flick the bottom of the tube to re-suspend the cells
Use approximately 6ml of media in the new flasks
Gently add and slowly pipette up and down in the tube. Pipette into the flask.
Label using ethanol resistant pen

General Lab

Creation of DMEM media containing 1% Penicillin Streptomycin, 10%FBS, and 1% Glutamine
Thawed HEK293T cells to create six cell lines -MRG

Creation of 100 mL cell culture media -JW

<u>Igem Preparation</u>
Creation of Liquid media samples of GFP colonies containing 1x CAM antibiotic
Creation of Dark box for presentation of GFP and RFP expressing *E.coli* cells -MRG

Transformed cells with RFP, GFP
I13521 plate 3 well G6 TetR-RFP
10uL water to well

Thawed competent cells DH5alpha on ice
1uL DNA to 50uL cells heat shocked 45 seconds 42 degree water then back on ice
added 200uL liquid LB to cells
shaker 1.25 hours spread on plates -GM

DNA extraction trials with grapes and blueberries
Transformation (LacI RFP) -JW

- Cell culture preparations
- Thawing/plating cells (HEK293T)
- Made media for cell culture
- DNA extraction trials: grapes, blueberries
    - Grapes: 4, 6 or more grapes
    - Blueberries: 6 berries

  Results: No DNA extracted from grapes in any trial; some DNA extracted from blueberries
- Transformation (fluorescent bacteria):

  J00450, LacI-RFP (2016 plate #6, well 12P)
  Competent cell: DH5α
  Plate: CAM plates

June 8, 2016

What we did today:
Split cells
Made and plated agar
Worked on survey/ printed poster
Transformed cells

Other team members:
Worked on bacterial plates
Made the "traits" wall art

Made 1 L of agar:
Mixed 40g LB agar with 1 L of water. Autoclave for an hour and a half, then let cool.
Add 1 mL/Liter of CAM

Expand Cells from T25 Flask (Done in hood):
Since most cells were happily growing, alive, and densely populated we decided to expand cells for reculturing instead of splitting them.
Removed all media from T25
5 mL of PBS was put into T25 flask and swirled to make sure it covered all cells. Then completely removed
0.5 mL of trypsin was added to the cells in the T25 flask and swirled around to make sure the cells were completely coated.
The flask was then knocked to unstick the cells from the flask.
5mL of media was then put into the flask with the cells and the trypsin and mixed by pipetting up and down vigorously.
The media and cells were then moved from the T25 to the bigger flask.
The cells were viewed in the new flask to make sure they were adequate and then stored in the incubator.

Transformation Protocol (Using InterLab Study):
Thaw HD5(alpha) cells on ice
50 µL cells/tube (5 tubes)
Add 1 µL DNA (spin before opening)
Sit on ice for 30 mins
Heat shock – 42°C water bath for 45 seconds
Put back on ice
200 µL LB liquid/tube
On shaker platform for 1-2 hours
Plate all to CAM plates
Plates incubate at 37°C

*Changes to Protocol with interlab samples:

Added 10 µL diH2O to samples with brown and light pink caps before putting with competent cells (could not get samples from tubes otherwise)

Splitting Cells
Checked incubated cells in flask under microscope. They were ~80% confluent and very densely grown in the middle of the plate. After sterilizing the hood with ethanol and UV light, we laid out: the flask with the cells,
Media (DMEM, Glutamine, PS, and FBS), PBS, and trypsin.
We first removed all of the old media, and disposed of it into the waste beaker. We then added 5 mL of PBS and gently swirled the flask before it was completely removed. .5 mL trypsin was added and swirled to cover the base of the plate. The flask was knocked while kept horizontal to unstick the cells from the base of the flask.
5mL of the media was added to the cells and trypsin, and vigorously pipetted up and down to break up the cell clumps. All of the liquid in the flask was then removed from the flask and added to a tube. 2 mL of the liquid in the tube was then added back to the flask. The remainder of the liquid in the tube was discarded in the waste beaker. The flask containing the media and the cells examined under a microscope to ensure the cells survived the splitting. After confirming the cells were successfully split, the flask was put back into the incubator.

Other tasks for the day:
Making agar
Plating agar into Pyrex dishes and square plates in preparation for Touch Tomorrow.

• Confluence: ~70%

• Split cell culture (1:3)

General Lab
Maintenance of HEK293T mammalian cell cultures

Cell culture split (1:3) -JW

Cells only about 30% confluent didn't split -GM

<u>IGem Preparation</u>
Creation of 1:10, 1:100, and 1:1000 dilutions of GFP *E.coli* in LB media and plated on CAM plates to test best dilution for creation of *E.coli* drawings
Creation of next generation of *E.coli* in LB media and 1x CAM
Creation of 3 RFP cultures in CAM, (2 with TetR-RFP and 1 with LACI-RFP)

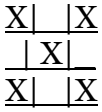
June 9, 2016

What we did today:
Counted cells in well
Colored with bacteria

Split Cells
Checked on Transformation

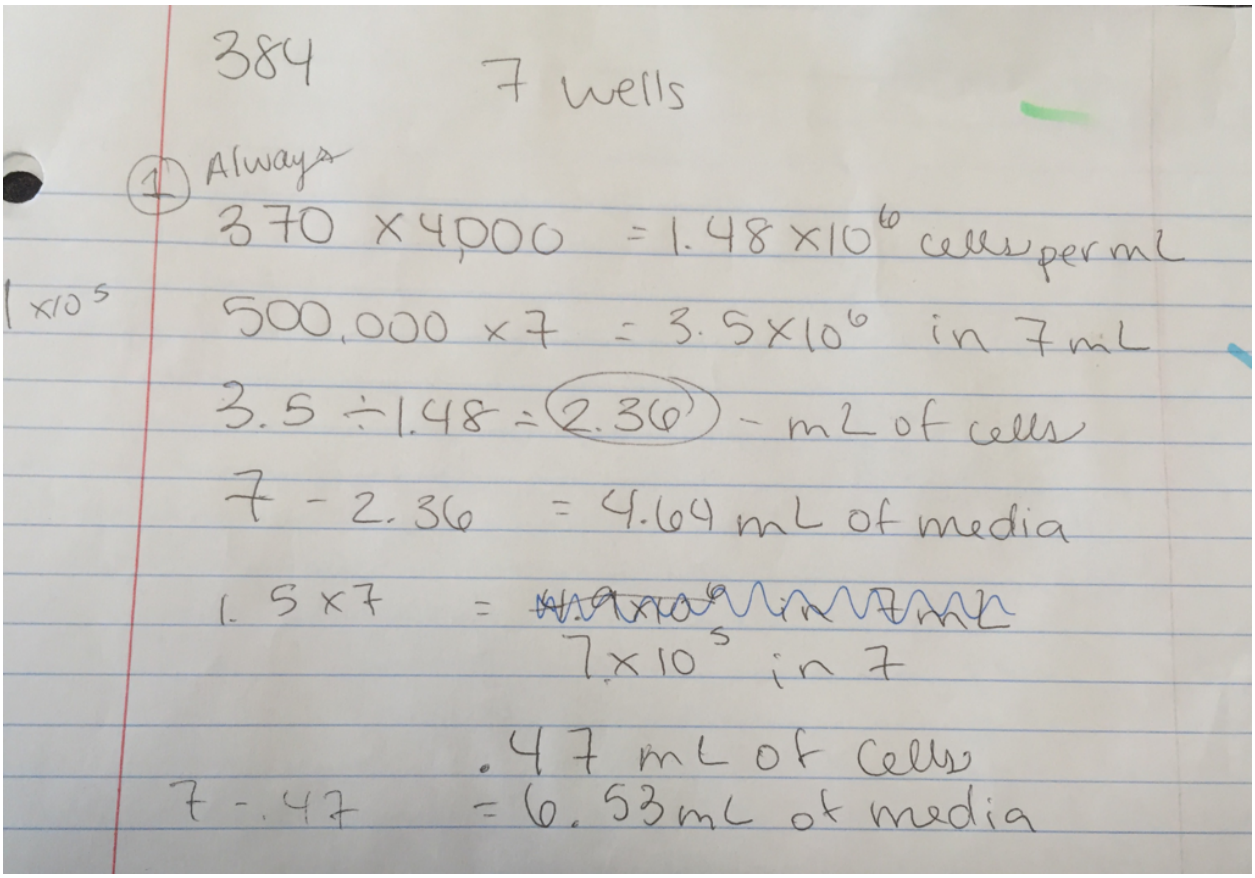
What other team members did today:
Split cells

Counting cells protocol:
Check cells to see if they need to be split. If not, put them back in the incubator. If so, split first. Instead of throwing away the liquid (cells, media, trace amounts of trypsin) in the tube, keep the liquid to use for cell counting.
Pipette 10 µL of the cells in media into microcentrifuge tube. Add 10 µL of trypan blue to the microcentifuge tube. Pipette up and down a few times to mix.
Take counting grid and cover, wipe down with ethanol. Place cover onto grid.
Take 10 µL of the mixture of cells and trypan blue and pipette in the middle of the entrance to the grid cover. Ensure there are no bubbles and the mix spread out to cover the grid.
Check under microscope. Count all the cells in the outmost corner boxes, and the middle box of the grid



Take total, multiply by 4,000. This is your count of cells / mL
Take the concentration you want to have, then multiply by the number of wells you want to fill+1
Take the second number and divide by the first number. This is the mL of cells you will want to use.
Subtract the number of mL of cells from the number of mLs you want to end up with (usually the number of wells you want to fill+1). This is the amount of media you need to reach your final volume.
Mix these amounts into a centrifuge tube and mix by inversion. Pipette 1 mL into each well. Incubate.

Ex.
370 cells x 4,000 = 4,000 1.48 x 10^6 cells / mL
500,000 x7 = 3,500,000 in 7 mL
3.5 / 1.48 = 2.3649 mL of cells mixture needed
7 - 2.36 = 4.64 mL of media needed



General Lab
Maintenance of HEK293T Cells
Creation of 1x10^5, 3x10^5, 5x10^5 cell plates for optimization testing

Cell count (Ginny's flask): $8*10^5$ cells/mL
Cells plated at $4*10^5$ cells/mL in 3 wells -JW

Split cells 1:4
Cell count $8*10^5$ cells/mL, cells plated at $2*10^5$ cells/mL in 6 wells - GM

<u>iGem Preparation</u>
Creation of fluorescent bacteria art for exhibition with 1:10 dilution liquid culture -JW

- Fluorescent bacteria art
  - Green: GFP colony 2
  - Red: LacI RFP colony 1
  - 1:10 dilution (50 µL bacteria liquid culture + 450 µL water)
  - Painted with toothpicks
- Cell count (Ginny's culture)
  - Total count: ~200 cells * 4000 = $8*10^5$ cells/mL
  - 1.75 mL cell + 1.75 mL media
  - Plate in 3 wells at $4*10^5$ cells/mL

June 10, 2016

What We Did Today:
Filled Isopropanol tubes
Tested extractions
Set up for TouchTomorrow
Trimmed DNA Origami
Put Up Remaining Posters
Checked and Photographed Cell Plating Experiments
Split Cells as Necessary

Sarah Cells: 40-50%

General Lab

Observed of confluence of differential cell density optimization
Maintenance of HEK293T cell cultures
Cell culture split (1:4) -JW

<u>iGem Preparation</u>
Creation of fluorescent bacteria art for exhibition with 1:10 dilution liquid culture -JW

- Confluence: ~90%
- Split cell culture (1:4)
- Bacteria art